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## PLAGUE AMONG THE GROUND SQUIRRELS OF CALIFORNIA.\*

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THE fact that a number of ground squirrels have been proven to be infected with *Bacillus pestis* in two widely separated sections of the state of California is perhaps the most serious feature of the plague situation in America. Ground squirrels of various and more or less closely related species may truly be said to be innumerable throughout the mountains and lowlands of the western states from Alaska to and beyond the Mexican boundary line. Hillsides, railroad cuts, river banks, and fields are literally perforated by their complicated systems of subterranean tunnels and innumerable inlets and outlets. Ranchers pray for the day when some efficient means for their extermination is found and their ravages cease. Of all the places in the earth "North America is richest in the *Arctomyinae*, her ground squirrels being numerous and diversified." Represented by the various rock squirrels of the Rockies, the prairie dogs of the western plains, and the groundhogs of the eastern states they reach from the Atlantic to the Pacific Ocean.

\* Received for publication October 26, 1908.

THE OCCURRENCE OF PLAGUE AMONG THE ARCTOMYINAE AND  
SCIURINAE ELSEWHERE IN THE WORLD.

"While tree squirrels (*Sciurinae*) abound in the torrid as well as in the temperate zone, the ground squirrels (*Arctomyinae*) are more northerly, on the whole being confined to temperate and colder latitudes, and next to America occur in greatest numbers in Asia."

Extending between 50° and 54° N. throughout the transbaikal region of Eastern Siberia and Northern Mongolia there exists a rodent larger than, but closely related to, our eastern "groundhog." The Mongols call them "tarbagans." In Russian they are called "beibak" and in German "Bobak" (*Arctomys bobac*, Schreb.). According to Rudenko<sup>1</sup> these animals dig long passages under the ground where they have their dens. They hibernate for seven months, usually from September 14 to March 15, and when they emerge in the spring they are much hunted for the sake of their flesh and fat. There are years when the tarbagans are swept by an epizootic which ceases by the end of summer. The sick animals will not go into their burrows, they are sleepy, crawl slowly, or stagger while running. When examined, a tense red swelling may be seen under the shoulder and this exudes dark fluid blood on section. They appear to be harmless to dogs when eaten and yet the Burjats, Cossacks, and Mongols fear these sick animals, as apparently their sickness may be transmitted to man, who then dies quickly. There is no reasonable doubt that this disease of tarbagans is plague, as Rudenko contends. One of Rudenko's accounts of human infection following the handling of sick tarbagans is particularly interesting:

In September, 1894, a number of deaths occurred in the family of the Cossack Mirsanoff, in the village of Sektuevsk. It seems that the father Mirsanoff had to go out of his village to Zaganolin to attend court. His pet dog trotting along beside him managed to kill six tarbagans in a very short time. Now the fact that the dog could kill six tarbagans while running by his master's side indicates that the animals must have been sick; healthy tarbagans are never caught under such conditions by a dog. Mirsanoff took the tarbagans along with him and later hid them in a hay pile in order to take them home on his way back; which he did on August 31. On September 2, he became ill and died on the 5th. As the illness was thought to be due to catching cold, no preventive measures were taken. In spite of the customary burial with a wake, none of the guests became ill. But six days later one of the sons became ill and died two days later, with manifest swelling of the glands in the axillary and

<sup>1</sup> *Russian Military Med. Jour.*, 1900, pp. 35, 67, reviewed by L. Heydenreich, "Die Pest der Tarbaganen," *Centralbl. f. Bakt.*, 1901, 29, p. 218.

inguinal regions. Now the family and neighbors recognized the danger. The family were strictly isolated in a vacant house, but a day or so later (September 15) two of the sons went into the forest after some wood and returned on the 18th, but one was brought home dead of the same malady. A daughter became ill on the 17th and died on the 21st. On the 19th still another son became ill and died on the 22d. The 80-year-old grandmother became ill on the 20th and died on the same day. On October 2 another daughter fell ill, became delirious, and died on the 3d.

And again according to Bannerman,<sup>1</sup> the common striped squirrel, *Sciurus palmarum*, so numerous in the verandahs and gardens of India, has been found to suffer from epidemic seizures of plague.

In December, 1898, a grey-striped squirrel was picked up dead at Cadag, in the southern portion of the Bombay presidency, where plague was at the time prevalent. There was no bubo in this animal but the spleen was enlarged and plague bacilli were isolated from its tissues.<sup>2</sup>

#### REVIEW OF THE EVENTS WHICH LED TO THE DISCOVERY OF PLAGUE AMONG SQUIRRELS IN CALIFORNIA.

I am greatly indebted to the generosity of P. A. Surgeon Rupert Blue, commanding plague-suppressive measures in San Francisco and vicinity, for data in this section, and I shall quote from his report. However, I believe it will be well to introduce some additional data which, along with reference to the accompanying relief map, will enable a reader unacquainted with local conditions to gain a proper orientation.

It will be remembered that, during the present pandemic of plague, the disease was first detected in San Francisco in 1900, when there were 22 fatal cases. These were practically limited to the district enclosing Chinatown. In 1901 there were 30 cases with 25 deaths in San Francisco. In 1902, 41 fatal cases and, in 1903, 17 fatal cases were reported from San Francisco. Now Dr. Blue reports:

While investigating the origin of one of the early cases of plague in San Francisco (Bock, 1903) I was impressed with the possibility of ground-squirrel infection in Contra Costa County. The efforts made at that time, however, to prove the hypothesis by finding an infected squirrel failed. Physicians and ranchers living in that section of the state, while apparently deeply interested in the subject, could not or would not devote sufficient time to the investigation. Two specimens only were obtained and in these putrefaction was too far advanced to admit of a bacteriological examination.

In 1903 it was rumored that an epizootic had destroyed the ground squirrels of the upper and middle portions of the county the year before. The report was con-

<sup>1</sup> *Jour. of Hyg.*, 1906, 6, p. 182.

<sup>2</sup> Dr. Alice Corthorn, "Plague in Monkeys and Squirrels," *Indian Med. Gazette*, March, 1899; referred to as above quoted by F. G. Clemow, "Remarks on Plague in the Lower Animals," *Brit. Med. Jour.*, 1900, 1, p. 1141.



MAP.—The stars in the region of Bay Point, which projects northward into Suisun Bay, indicate the localities where plague-infected squirrels were found. The star with a white center indicates the location of the Faria Ranch, where a boy, J. F., died of plague on July 15, and where a squirrel dead of plague was found, on August 5.

firmed by several inspections which were made with the view of defining the boundaries of the infected area, and to obtain sick or dead squirrels. About this time (August, 1903) a country blacksmith, Charles Bock by name, of Pacheco, Contra Costa County, came to San Francisco and died of plague in the German Hospital. In order to investigate his history I visited Pacheco, Cal., and while there was informed that Bock had shot ground squirrels three or four days before his illness began. He had not in the past thirty days visited Oakland or San Francisco.

On September 13, 1903, E. T. S., a bridge-builder, employed by the Southern Pacific Company, in construction work near Danville, Contra Costa County, died of bubo-septicemic plague, in the Southern Pacific Hospital in San Francisco. He had lived for the past two months in a railroad camp in the San Ramon Valley, thirty miles away from the centers of population. Ground squirrels had often been killed and eaten as a delicacy by the rough laborers.

In 1904, nine cases of plague with eight deaths were recorded in San Francisco. With regard to the occurrence of cases outside of San Francisco, Dr. Blue reports:

In February, 1904, Dr. F. F. Neff, a practicing physician of Contra Costa County, reported a suspicious case in a woman living near Concord, Cal. P. A. Surgeon B. J. Lloyd, then on duty at the Plague Laboratory, Chinatown, was detailed to investigate the case. He reported that the patient, Mrs. F. S., had died of bubo-septicemic plague soon after his arrival at her home. Some of the glands were excised and examined at the plague laboratory and showed typical gross and microscopical findings of plague.

P. A. Surgeon Donald Currie, who was then bacteriologist for the plague laboratory, carried on a series of experiments with a view to ascertaining the susceptibility of the ground squirrel to plague infection, by inoculation and direct contact. This work was done in the summer of 1904 and gave positive results in both cases. Some three dozen squirrels were used in the experiments. The animals were obtained through an advertisement inserted in a daily paper, being trapped in San Mateo County.

In September, 1904, a thorough inspection was made of Contra Costa County, for the purpose of locating accurately the sections covered by the rodent epizootic of 1902-3. Dr. Medros, acting under my direction, spent a week driving through the country and interviewing the farmers in regard to the disappearance of rodents. He ascertained that the epizootic began on the water front at Port Costa in 1903, and extended southward to Walnut Creek and eastward to Antioch in a year. In this triangular section the squirrels had almost entirely disappeared. Only a few of the people interviewed had seen sick or dead animals, but all had noticed the rapidly diminishing numbers. It was generally believed that the mortality was due to a "contagious disease" which had been started among them by the professors of the University of California. The experiments of Dr. Currie, however, disproved this theory, as squirrels are not susceptible to infection by Danysz, or to any of the viruses in common use.

The authorities at the State University denied that any such attempt to exterminate squirrels had been undertaken by any of their staff, when inquiries were made by Passed Assistant Surgeon J. D. Long and myself.

Passed Assistant Surgeon J. D. Long, until recently in charge of the situation in Alameda and Contra Costa counties, made inquiries concerning past epizootics among the ground squirrels and learned from Mr. Hook, a supervisor of Contra Costa County, that in the region of Martinez, between 1903 and 1905, on several occasions, great numbers of squirrels were seen to migrate across the country, so that in places where they had been numerous they became scarce and vice versa; and in the neighborhood of Livermore, Alameda County, a rancher who appeared to be a keen observer had given similar accounts and had added that persons came into the region where sick squirrels were dying, and had taken sick squirrels elsewhere in order that the disease might spread. At one time Dr. N. K. Foster, secretary of the State Board of Health, offered as much as \$20.00 for a sick squirrel but was unable to obtain one.

In 1905 and 1906 no cases of plague were observed in San Francisco. Dr. Blue further reports on the status of affairs in Contra Costa County:

There were no suspicious cases reported in the summer of 1904, or in 1905, although a careful watch had been maintained by Dr. Neff and others in the county. In March of 1906, however, a boy sickened in East Oakland with typical bubonic plague. The investigation which followed failed to connect the boy with a previous case of human plague, but showed that he had shot and eaten ground squirrels three or four days prior to his seizure. The squirrels were killed in Strawberry Canyon, which is about half a mile east of the grounds of the University of California.

In 1907, after the earthquake, plague again appeared in San Francisco. The first case detected was in a sailor, from the tug-boat "Wizard," who died at the Marine Hospital at San Francisco, and was diagnosed by Passed Assistant Surgeon J. D. Long. During the year 156 cases with 78 deaths occurred in San Francisco. Ten cases were reported from Oakland. The last case of human plague in San Francisco was on January 30, 1908, and the last infected rat found in that city was found on July 28, 1908. From all over the city 30,880 rats have been examined since that time, with negative results. The last case of human plague (excepting one) in Oakland was found on December 22. A number of infected rats were found in this city in the spring months, but none between April 15 and July 15 (however, only 9,361 rats were examined). For the history of

the last case found in Oakland (July 21, 1908) I would refer the reader to the last section of this article.

Now, a boy, J. F., died July 15 on a ranch out of Concord, Contra Costa County (see star with white center on map). He was reported as suspicious by Dr. DeWitt, county health officer, and autopsied by Dr. W. H. Harrison, Jr., state inspector. It was subsequently proven by laboratory methods that the boy had died of plague. And on July 28, another case (M. P.) was found in this county, in Briones Valley, about 10 miles from Martinez. Investigations by Dr. Foster, Dr. Long, Dr. Rucker, and Dr. Howe revealed the fact that there had been a mortality among the rats on a ranch near the place where the boy, J. F., died. Here a rat was found dead and turned over to Passed Assistant Surgeon McCoy, who found that it was anatomically suspicious for plague, but decomposition was too far advanced to prove it to be plague by animal inoculation. Dr. Blue directed that men be sent from Dr. Long's force in Oakland to trap and collect rodents in Contra Costa County. Between August 5 and October 10, 423 ground squirrels, 82 rats, 3 mice, 18 jack-rabbits, 2 chickens, 1 gopher, 1 ground owl, and 1 coyote have been received for examination at the Oakland Plague Laboratory of the U. S. P. H. and M. H. S. Most of these animals were shot. Four out of the 423 ground squirrels were infected with *B. pestis*, as detailed in the next section of this article. Three of the four were found dead. Number 1 was found dead on August 5, near a barn on the Faria ranch, where the boy, J. F., died on July 15. No other evidence of any disease which might explain the histories of past epizootics among the animals in this region was obtained.

The results of Dr. Currie's experimental work with ground squirrels combined with the epidemiological data accumulated convinced many that the ground squirrels were a not infrequent source of human infection. It is only fair to state here that I, personally, was skeptical of its importance. However, the history of the case which occurred in Los Angeles, combined with the observations of Dr. Currie that a considerable percentage of inoculated squirrels develop plague pneumonia, and the fact that the mucus in the posterior nares of squirrel No. 1 (*vide infra*) was proven to be infectious, leaves no room for doubt that the bite of a plague-sick squirrel may result in



infection. For the history of the Los Angeles case I am indebted to Passed Assistant Surgeon Geo. W. McCoy, who was detailed to investigate the case. He reports as follows:

I arrived in Los Angeles on August 30, 1908. Surgeon Brooks, Public Health and Marine-Hospital Service, and Dr. Powers, the health officer, met me at the train and after a short preliminary conference at Dr. Brooks's office, we visited the case. The patient was a boy ten years old, who lived with his parents at Elysian Park in the northeastern part of the city, about two miles from the city hall. This park adjoins the yards of the San Francisco-Los Angeles line of the Southern Pacific Railroad.

Several days prior to the onset of his illness, the boy found a ground squirrel near his home. The animal appeared sick and made no effort to escape from the boy when he picked it up, but it bit him on one of the fingers of the right hand. Just what became of this squirrel is uncertain. It is believed to have been killed by a cat or a dog. Four or five days later, on August 11, the boy became ill with a headache, delirium, and fever which rapidly rose to 106° F. and for several days he was quite sick. On the second or third day of the illness the right axillary glands became swollen and painful and later the other superficial glands became enlarged.

The boy was under the professional care of Dr. E. B. Alexander. Dr. Alexander called Health Officer Powers to see the case and later Surgeon Brooks was asked to see it. All of these gentlemen had had experience with plague and all regarded this case as decidedly suspicious.

On August 20, the right axillary gland was aspirated and a few drops of bloody fluid obtained. Nothing was to be seen in the smears, but a culture was made in broth which gave a growth that was regarded as a streptococcus, but from the appearance of the stained specimens from this culture submitted to me for examination I am convinced that the organism was a bacillus growing in chains. This culture was turned over to me and after making a careful study of its growth on artificial media and conducting inoculation experiments on animals (rats and guinea-pigs) I have demonstrated that the organism is *B. pestis*. When I saw the boy on August 30, he was without fever and appeared to be convalescent, but was very weak. The right femoral gland had been incised and there was a free discharge of pus. The right axillary glands were much enlarged. There was great infiltration of the surrounding tissue and softening beginning at one point. The history, with the appearance of the case at the time I saw it, compelled me to share the suspicions of those who had seen it earlier.

On August 21 a squirrel was found dead within 50 yards of the place where the boy had been bitten. There were no signs of injury and a post-mortem examination by Dr. Black, professor of bacteriology in the local medical college, showed an inguinal bubo and large spleen. This bubo and the spleen were used to inoculate a guinea-pig by the cutaneous method. The guinea-pig was found dead on the morning of August 28. The guinea-pig had been kept in Kaiserling's solution for my examination. It presented a caseous bubo, a dark liver with many whitish granules, and a large spleen full of whitish granules, in other words, the typical appearance of plague in the guinea-pig. Smears made from the liver and spleen by Dr. Black showed an abundance of bipolar bacilli and coccoid forms. A culture was made from the heart's blood. This culture was turned over to me for study and it answers all of the requirements for *B. pestis*. It should especially be mentioned that it gives charac-

teristic growth on agar and in broth and gives characteristic involution forms in 24 hours on salt agar. White rats, wild rats (*Mus norvegicus*), and guinea-pigs were inoculated with this culture, and all reacted with the typical lesions of plague.

I made post-mortem examinations of several squirrels and two cats, all killed or found dead in the vicinity where the dead squirrel was found. All were negative for plague.

#### THE ANATOMIC AND BACTERIOLOGIC FEATURES OF SQUIRREL PLAGUE AS OBSERVED IN CALIFORNIA.

All of the ground squirrels examined up to date have been of one species, *Otospermophilus beecheyi* (Richardson).<sup>1</sup>

*Squirrel No. 1.*—Found dead on the Faria Ranch, and received for examination on August 6, 1908.

On dissection the subcutaneous tissues showed some injection; the spleen and liver were somewhat enlarged, dark, and soft; there was a small amount of clear fluid in the thoracic cavity; there were areas of consolidation in the lungs. Upon the removal of thoracic organs *in toto* the anterior lobes of both lungs showed grayish areas of consolidation, involving the whole anterior lobe and anterior portion of the posterior lobe on the left side and the anterior and middle lobes and part of the posterior lobe on the right side. The grayish areas were surrounded by deeply congested zones and without the areas of pneumonia there were quite a number of subpleural petechiae. The involved tissues appeared grayish red on section, and exuded an anchovy-sauce-like material upon pressure. (See Plate 4, Fig. 1.)

<sup>1</sup> The following description of this species is given by E. A. Mearns, M.D., major and surgeon U. S. Army, in "Mammals of the Mexican Boundary of the United States," *Bull. 56* (1907) *U. S. National Museum*, pp. 314 ff.

#### *Otospermophilus beecheyi* (Richardson) CALIFORNIA GROUND SQUIRREL

Type-locality.—The neighborhood of San Francisco and Monterey, in California.

*Description.*—Size smaller than *Otospermophilus grammurus* (nearly as large as the eastern gray squirrel) with a more slender body and shorter tail. Ears high and pointed. Mammary glands, six pairs (P. 2/2, A. 2/2, I. 2/2=12). Color above brown, grizzled and annulated with black in a vermicular pattern; darkest anteriorly, and most grizzled and vermiculated posteriorly. Nape and sides of neck silver gray, this color prolonged backward above the shoulder in the form of stripes which are sometimes faintly traceable to the root of the tail, though usually ending about the middle of the body. Ears black outside, grayish, or faintly rusty inside, and along posterior border. Top of head bistre, slightly dusky above orbits, which are encircled by white. Sides of head grayish, mixed with yellowish brown. The tail, which is less bushy and shorter than in *O. grammurus*, is yellowish gray, the lateral hairs thrice annulated with black. Feet yellowish gray. Under surface of body grayish white. The inter-scapular region is often blackish, more or less vermiculated with pale annuli. Length, 410 mm.; tail vertebrae, 170; hind foot, 55; ear above crown, 21; ear above notch, 27; length of head, 62; skull, 57 by 34 mm. (averages).

Major Mearns observed that young were being born during the whole period of collection from May 10 to July 13, 1894. In coloration they closely resembled their parents at the same season. I have obtained some specimens larger than those obtained by Major Mearns.

Further examination revealed an ulcer, about 10 mm. in diameter on the back, just anterior to the root of the tail. On section the tissues beneath the ulcer showed congestion. This subcutaneous congestion was traced to an abscess about  $15 \times 10 \times 10$  mm. apparently occupying the position of the left inguinal glands. The abscess was full of a pale greenish-yellow, viscous, purulent material.

*Microscopic examinations.*—Smears from the consolidated areas in the lung showed enormous numbers of bipolar-staining rods and coccoid forms resembling *B. pestis* (Carbol Thionin). These were also very numerous in the mucus of the posterior nares and in the subcutaneous tissues just beneath the cutaneous ulcer. Numerous bipolar-staining bacilli resembling *B. pestis* were seen in smears of the pus from the inguinal abscess. Smears from the spleen and heart's blood showed only a few rods and these did not present a good bipolar appearance. Micrometric determinations (Zeiss, oil imm., oc. microm. 6) showed that the bipolar-staining rods in the lung smears varied from  $1.4\mu \times 1.2\mu$  up to  $1.6\mu \times 1.2\mu$  while the coccoid forms were generally  $1\mu$ – $1.2\mu$  in diameter. Comparative measurements made at the same time showed that the bipolar-rod forms of the bacillus of rabbit septicemia were  $1\mu \times 0.5\mu$  and the coccoid forms  $0.4\mu$ – $0.6\mu$  in diameter.

*Animal inoculations.*—A white rat was inoculated cutaneously by rubbing a piece of the consolidated pulmonary tissue into a scratch on its abdomen. It died in a little less than three days, showing marked subcutaneous congestion, hydrothorax and congestion of all the organs. Bacilli resembling *B. pestis* were few in the inguinal glands, but very numerous in the spleen, and were obtained in pure culture.

Guinea-pigs Nos. 1 and 2, inoculated cutaneously with pulmonary tissue, died after eight and seven days respectively, and showed marked subcutaneous injection, large hemorrhagic inguinal buboes, hydrothorax and multiple ecchymoses beneath the serosa of the lungs and large intestines. There were numerous bipolar-staining rods resembling *B. pestis* in preparations from the buboes and spleens. Cultures resembling plague were obtained from the spleen and heart's blood of each in purity.

Guinea-pig No. 3, inoculated cutaneously with a little of the mucus from the squirrel's posterior nares, died in seven days with the same anatomical changes as in guinea-pigs Nos. 1 and 2. Pure cultures resembling plague were isolated from its spleen and heart's blood.

Guinea-pig No. 4, inoculated cutaneously with purulent material from the inguinal abscess, died in five days with the above-described anatomical changes. Pure cultures resembling *B. pestis* were isolated from its spleen.

*Cultural studies.*—+1 agar slants were inoculated from the lung, spleen, and heart's blood of the squirrel. In 24 hours at  $37^{\circ}$  C. these showed numerous minute transparent colonies which were viscous. These growths yielded typical large club-shaped and yeast-like involution forms on 3 per cent salt agar after 24 hours' growth at  $37^{\circ}$  C.

The cultural characters of the bacilli isolated from the lung of the squirrel itself and from the spleens of white rat No. 1, and guinea-pigs Nos. 1, 2, 3, and 4 may be summed up as follows: They corresponded with *B. pestis* in that growths on +1 agar slants resembled those of *B. pestis*, and were viscous; they all formed large clubbed and yeast-like involution forms on +1.3 per cent salt agar in 24 hours at  $37^{\circ}$  C.; they grew as a stringy, viscous mass at the bottom of the test-tube in +1 sugar free bouillon;

they produced no visible change in + 1 milk; they grew delicately in and did not liquefy + 1.5 gelatin at 18-22° C.; they produced acid but no gas in + 1 broth containing 1 per cent of dextrose, levulose, galactose, maltose, and mannite, but no acid nor gas from lactose, saccharose, nor inulin. The culture from the heart's blood of white rat No. 1 produced typical stalactites in + 1 oiled broth.

A piece of the squirrel's lung was sent to Passed Assistant Surgeon Geo. W. McCoy for confirmatory diagnosis. His report has already been published.<sup>1</sup> McCoy carried out a series of valuable experiments, some of which I quote:

While no doubt was entertained as to the nature of the organism under investigation, it seemed desirable to test it against antipest serum. For this purpose we used guinea-pigs and rats (*Mus norvegicus*). The culture was the same as in the above experiment (direct from the squirrel's lung), but was the third generation, and the growth was 48 hours old. In each case the animal was given subcutaneously 0.01 of a loopful of the agar culture. Three guinea-pigs were used.

The first guinea-pig was given 5 c.c. of antipest serum intraperitoneally just prior to the inoculation. This animal never sickened, and is alive and well at the present time, 10 days after the inoculation.

The second guinea-pig was given 5 c.c. of antidiphtheria serum intraperitoneally just prior to inoculation. This was done to provide a serum control. This animal died on the fourth day with characteristic lesions of plague, and cultures from the liver gave a pure culture of *Bacillus pestis*.

The third guinea-pig was inoculated with the culture in the same manner as the previous ones, but no serum was given; therefore it served as a control. This guinea-pig died on the fourth day and presented lesions characteristic of plague. An organism agreeing with the one isolated directly from the squirrel was obtained in pure culture from its liver.

Four rats (*Mus norvegicus*) were inoculated with the same amount of the same culture as was given to the guinea-pigs. Two of the rats that had previously been given 5 c.c. of antipest serum were killed on the eighth day and were shown by post-mortem examination to be normal. The two that had not received serum (controls) died on the fourth day and both presented typical lesions of plague as seen in the rats, and a pure culture of *Bacillus pestis* was isolated from the liver in each case.

In order to determine whether the organism was as virulent as the *Bacillus pestis* isolated from rats here, two series of guinea-pigs were inoculated with the results shown in the table below. An agar culture was used in each case.

QUALITY OF CULTURE	GUINEA-PIG DIED	
	Squirrel Second Generation	Rat No. 66 (Fourth Generation)
1 loopful, vaccinated . . . . .	Fourth day	Fifth day
0.01 loopful, inoculated, subcutaneously . . . . .	Fifth "	Third "
0.001 loopful, inoculated, subcutaneously . . . . .	" "	Seventh "
0.0001 loopful, inoculated, subcutaneously . . . . .	Eighth "	Sixth "
0.00001 loopful, inoculated, subcutaneously . . . . .	Fifth "	Seventh "
0.000001 loopful, inoculated, subcutaneously . . . . .	Seventh "	Sixteenth "

<sup>1</sup> *Public Health Reports*, 1908, 23, p. 1289.

The control was *Bacillus pestis*, isolated from plague rat No. 66. Neither of these cultures had ever been through an animal other than the ones in which they were found in nature. It will be observed that there is no material difference in the virulence of the cultures.

*Squirrel No. 2.*—Found dead on the Morton ranch, Contra Costa County, on August 21, 1908. Received for examination August 22.

The body showed marked post-mortem decomposition and was badly fly-blown. There was an abscess  $8 \times 5 \times 5$  mm. in the right inguinal region and what remained of the left axillary gland indicated that it might have been caseous. The lungs were badly macerated but a small area was found which was of firmer consistency than the surrounding pulmonary tissue. Smears from the lung nodule and axillary gland showed a number of very faintly stained yeast-like bodies about the size of the coccoid form of *B. pestis* along with numerous postmortem invaders. Smears from the inguinal abscess showed no bacteria, excepting some large postmortem invaders.

*Animal inoculations.*—A white rat, inoculated cutaneously with juice from the apparently consolidated portion of the squirrel's lung, was chloroformed in a dying condition on the 5th day. There was slight subcutaneous injection; no local reaction no buboes. The spleen was congested and soft. Pure cultures resembling *B. pestis* and which gave typical large clubbed involution forms on 3 per cent salt agar were obtained from the heart's blood and a similar culture was obtained from the spleen, though this was mixed with micrococci.

A guinea-pig inoculated cutaneously with lung tissue died in eight days with marked subcutaneous injection; large hemorrhagic and necrotic inguinal buboes; numerous minute areas of pneumonia in its lungs and foci of necrosis in the liver and spleen; the spleen was soft and congested. A pure culture resembling *B. pestis* was obtained from its spleen. This culture produced typical stalactites in oiled broth. A guinea-pig inoculated cutaneously from the squirrel's axillary gland died in eight days with the same anatomical findings described in the above pig. A culture from its spleen was sent to Passed Assistant Surgeon Geo. W. McCoy for confirmation.

*Squirrel No. 3.*—Shot on the Southern Pacific railroad tracks near the Morton Foundry, Contra Costa County, on August 25, 1908. Received for examination August 26.

The body was in fresh condition. There was an encapsulated abscess about  $10 \times 5 \times 5$  mm. in the left groin; the lungs showed hemorrhagic areas due to shot and appeared normal, excepting a yellowish white consolidated area about  $4 \times 4$  mm., circular and extending about 4 mm. into the substance of the middle portion of the ventral surface of the left anterior lobe.

The inguinal bubo contained a creamy pus which showed a few bipolar-staining rods about the size of *B. pestis* along with other larger rods. Smears from the pneumonic area in the lung showed a few bipolar-staining rods and coccoid forms resembling *B. pestis* along with numerous postmortem invaders.

*Animal inoculations.*—A white rat inoculated cutaneously with lung tissue died in three days. It showed subcutaneous injection and congestion of the internal organs. The spleen was dark and congested. Smears from the spleen and liver showed numerous bipolar-staining bacilli resembling *B. pestis*. Numerous colonies resembling those of *B. pestis* were obtained from the spleen and heart's blood on +1 agar slants and transplants gave typical involution forms on 3 per cent salt agar in 24 hours at 37° C. The culture from its heart's blood produced typical stalactites in oiled broth.

A guinea-pig inoculated cutaneously with lung tissue died in seven days, with the typical lesions of plague. Pure cultures resembling plague were obtained from its spleen and heart's blood.

*Squirrel No. 8.*—Found dead in a field near Bay Point, Contra Costa County, by Passed Assistant Surgeon W. C. Rucker on September 19, 1908. Received for examination September 20.

There was some postmortem decomposition; no subcutaneous injection; no enlargement or congestion of the cervical, axillary, inguinal, abdominal, or pelvic glands; thoracic cavity almost full of a clear serous exudate; lungs appeared normal, excepting two petechiae about 1 mm. in diameter on the dorsal surface of the left anterior lobe; the spleen was firm and slightly enlarged, of a very dark reddish, blue-black color and of irregular contour, owing to the presence of numerous irregular nodules. These irregularly rounded nodular areas were 2–4 mm. in diameter and of a yellowish-gray color with hyperemic borders (see Plate 4, Fig. 2). They were very numerous at both ends of the spleen where they became confluent. On section, these nodules were seen to extend deeply into the substance of the organ and were composed of a firm yellowish-white tissue. The liver was of a chrome-yellow color, due to phosphorus or arsenic (?); its capsule was smooth and of an oily luster. Scattered over its surface, about 25 irregularly rounded reddish areas 2–3 mm. in diameter were seen. These reddish areas were especially congested at their peripheries. Besides these, there was an equal number of yellowish areas 2–3 mm. in diameter which in some cases projected slightly from, in others receded from, the capsular surface. On opening one of those yellowish areas it was seen to contain a yellowish purulent

sticky material. There were four similar yellowish areas apparently embedded in the muscular substance of the diaphragm.

*Microscopic examinations.*—In smear preparations from the spleen nodules there was quite a number of coccoid bodies and fewer bipolar-staining rods resembling *B. pestis*. There was also quite a number of large rod-shaped post-mortem invaders.

In preparations of the pus from a small abscess near the costal border of the liver a few bipolar-staining rods resembling *B. pestis* were seen. No bacteria were found in lung smears.

*Animal inoculations.*—Guinea-pig No. 1. was inoculated cutaneously with tissue from a spleen nodule; appeared very ill for five days, preceding death on the eighth day; some subcutaneous injection; inguinal glands large and rather pale on section; spleen greatly enlarged, about six times normal size, and full of minute whitish foci of necrosis; liver swollen and full of similar minute granular foci of necrosis; lungs showed numerous grayish tubercular nodules, 1–3 mm. in diameter, scattered throughout the substance and beneath the plurae of all lobes. All these nodules were surrounded by hemorrhagic zones, like the nodules in the squirrel's spleen. Rods resembling *B. pestis* and coccoid forms were very numerous in its spleen and lungs but very few were found in the inguinal buboes.

Guinea-pig No. 2, was inoculated cutaneously with pus from a small abscess in the liver. It ate its food regularly though it appeared ill for about eight days preceding its death on the 15th day. It showed very slight subcutaneous injection and large inguinal buboes 15×10×10 mm. surrounded by a slightly congested zone and some periglandular serogelatinous exudate. On section the buboes appeared caseous. The spleen was of about normal size and contained 20 or 30 irregularly rounded whitish nodules resembling those in the squirrel's spleen, though smaller; the liver showed nothing noteworthy; there was a clear serous hydrothorax; the lungs were intensely congested and at the apex of the left anterior lobe was a yellowish-white area of consolidation; another irregularly rounded area of consolidation 10×6 mm. occurred in the dorsal center of the right middle lobe and still another 12×10 mm. near the posterior margin of the right posterior lobe. All these pneumonic areas were surrounded by zones of intensely congested pulmonary tissue resembling the areas described in the lungs of squirrel No. 1. Microscopically bacilli resembling *B. pestis* were found in greatest numbers in preparations from the pneumonic areas in the lungs. Pure cultures resembling *B. pestis* were obtained from the lungs, heart's blood, and spleen.

White rat No. 1 was inoculated cutaneously from the spleen of guinea-pig No. 1. It died of acute plague in three days, showing marked subcutaneous injection, and congestion of the internal organs. There were no pulmonary lesions. Bipolar-staining bacilli resembling *B. pestis* were very numerous in the spleen and heart's blood. Cultures resembled those of *B. pestis*.

Guinea-pig No. 3 was inoculated cutaneously from the spleen of guinea-pig No. 1, in order to see whether the character of the lesions would persist; it died on the tenth day, showing slight local reaction; large pale inguinal buboes. On section the buboes showed caseous centers with a narrow zone of congestion about the caseous material. The lungs were very intensely congested and all the lobes contained many irregularly rounded, yellowish-white nodules 2–4 mm. in diameter, which were firm on section. There were also numerous similar nodules  $\frac{1}{2}$ –1 mm. in the liver and the spleen was literally full of yellowish-white irregular nodules, 2–5 mm. in diameter, resembling those

in the spleen of squirrel No. 8. Bipolar-staining rods resembling *B. pestis* were scarce in the inguinal buboes but numerous in the lung nodules.

Guinea-pig No. 4 was inoculated cutaneously from the spleen of white rat No. 1 in order to see whether the virulence for guinea-pigs was increased. It died in six days showing marked subcutaneous injection; large caseous inguinal buboes surrounded by a hemorrhagic and serogelatinous exudate; a spleen about twice the normal size, soft and full of yellowish foci of necrosis; lungs not consolidated. Rods resembling *B. pestis* numerous in spleen and inguinal buboes.

White rat No. 2 was inoculated cutaneously with a piece of consolidated lung tissue from guinea-pig No. 2. It died in two and a half days, showing marked subcutaneous injection; congestion of the internal organs; hydrothorax; but no pneumonia. Rods resembling *B. pestis* were very numerous in the spleen and inguinal glands.

Guinea-pig No. 5 was inoculated cutaneously with consolidated lung tissue from guinea-pig No. 2 to see if the tendency to localize in the lungs would persist. It died in five days and showed large hemorrhagic inguinal buboes with caseous centers. Its spleen was about four times the normal size, soft and full of yellowish-white foci of necrosis. The liver was congested and showed numerous foci of necrosis. Its lungs were greatly congested and showed areas of hemorrhagic consolidation and many yellowish-white consolidated areas 1-3 mm. in diameter and many subpleural petechiae. Bipolar-staining bacilli resembling *B. pestis* were very numerous in the lungs, spleen, and buboes.

*Cultural studies.*—Cultures on +1 agar slants from the spleen nodules of squirrel No. 8 yielded numerous delicate, viscous colonies which when transplanted to artificial media showed the same cultural characters described for *B. pestis* under squirrel No. 1. Besides the fine colonies there grew a number of large white colonies of an actively motile bacillus which belonged to the colon group—coagulating milk and fermenting dextrose, levulose, lactose, saccharose, maltose, galactose, and mannite with gas-production. Cultures from the heart's blood of squirrel No. 8. yielded a few scattered delicate plague-like colonies.

#### REMARKS ON CERTAIN FEATURES OF NATURAL SQUIRREL PLAGUE. THE EXPERIMENTAL WORK OF DR. CURRIE.

It should be noted that in three of the four naturally infected squirrels, the lungs showed areas of consolidation due to *B. pestis*. In the case of squirrel No. 1, which showed the most marked pneumonia, the mucus of the posterior nares was proven to be infective. Then it will be noted that in the series of animal inoculations, under squirrel No. 8, the tendency for *B. pestis* to localize in the lungs was shown by a series of cutaneous passages through guinea-pigs. However, when this strain was passed through a white rat, which died of acute plague, and then back into a guinea-pig, this guinea-pig died with the anatomical and microscopical findings of acute plague. I do not know whether such a tendency to localize in the lungs has been observed in natural rat plague. However, it is stated that "Martini



was able to produce plague pneumonia in rats in 32 out of 36 inhalation experiments and thought that the virulence was exalted by passage from lung to lung; and when such cultures were injected intraperitoneally or subcutaneously they showed a great tendency to localize in the lungs."

It seems quite possible that human pneumonic plague may be due to infection with a strain of *B. pestis* having a tendency to localize in the lungs. On the other hand this tendency may be the expression of a greater resistance, for Klein<sup>1</sup> found that a certain number of white rats, guinea-pigs, and monkeys, insufficiently protected by Haffkine's prophylactic, developed plague pneumonia. He suggests that the first human case may arise in this way.

This peculiar tendency to localize in certain sites is well illustrated by a human case, H. 171, which was autopsied last winter in San Francisco. The only lesions at autopsy were a number of tumor-like nodules in the liver containing bipolar-staining bacilli. A culture from one of these nodules was kept by me and later turned over to Passed Assistant Surgeon George W. McCoy who proved it to be a virulent strain of plague.

My assistant, Mr. A. Venzke, who was with Passed Assistant Surgeon D. H. Currie in 1904, called my attention to the fact that Dr. Currie often noted pneumonia in his experimentally infected squirrels. These squirrels were identical with *O. beecheyi* according to Venzke. Through the kindness of Dr. Blue I am able to refer to Dr. Currie's experiments, which have never been published.

Out of nine experiments, we can draw the following data: When ground squirrels are inoculated subcutaneously or cutaneously with plague cultures or the organs of guinea-pigs or squirrels dead of plague, they die in from 44 hours to 10 days. Leaving out the squirrel which lasted for 10 days, the average time till death is 3.75 days. Among these inoculated squirrels, No. 4 died of primary plague pneumonia without noticeable septicemia; and No. 8, which lasted 10 days, died of bubonic, pneumonic, and septicemic plague.

Further, Dr. Currie experimented on transmission by "contact." Out of six squirrels associated with plague-sick squirrels, three died of plague, one of primary plague pneumonia, and the other two of

<sup>1</sup> *Bacteriology and Etiology of Oriental Plague*, 1906, p. 108.

pneumonic and septicemic plague. Another of the contacts was chloroformed and showed a plague bubo in the axilla.

In a single feeding experiment in which two squirrels were fed on carrot upon which a few drops of blood containing plague bacilli had been smeared, one died in four days with a bubo just anterior to and under the angle of the lower jaw with secondary plague septicemia.

I will quote one of the experiments as reported by Currie:

*Squirrels Nos. 14, 15, and 16.*—October 22, inoculated No. 14 by dermic method (that is, by rubbing the infectious material on the shaven but unbroken skin of the animal) in the skin of the back, using the spleen of a guinea-pig which had died from inoculation with No. 9. Placed No. 14 in cage with two healthy squirrels, Nos. 15 and 16. October 25 No. 14 died. Necropsy showed an extreme degree of plague dermatitis at site of inoculation, which had gone on to gangrene. The tissue showed necrosis and sloughing which in some places had exposed the spinal column. The neighboring glands were enlarged and inflamed and there was a plague septicemia. October 31 contact squirrel No. 15 died. Necropsy showed a plague dermatitis about the nostrils and double pneumonia due to the plague bacillus and a moderate plague septicemia. No glandular enlargements were present. November 7 contact squirrel No. 16 died (13 days after last contact with No. 14 and eight days after last contact with No. 15). Necropsy showed primary plague pneumonia. There were a few bacilli in the spleen and heart's blood.

#### THE RELATION OF PLAGUE IN SQUIRRELS TO THAT IN RATS AND MEN.

Apart from the danger of the gradual spread of squirrel plague across country through these all too numerous rodents and apart from the occasional transfer of plague from squirrel to man, it seems altogether likely that ground squirrels may act as a host for the *Bacillus pestis* in the interim between the more noticeable outbreaks in rats and men. Plague kept alive in ground squirrels might then be reintroduced into more thickly populated sections either by human or rat carriers. For example, a human having acquired infection during squirrel-hunting might reintroduce the infection among rats either in the form of plague-infested squirrel fleas or by himself, being then the source of infestation for human fleas. *P. irritans*, the human flea, has been found, sometimes in considerable numbers, on rats (*M. norvegicus*) on both sides of the bay.<sup>1</sup> As will be shown

<sup>1</sup> By M. B. Mitzmain, *Monthly Bull. California State Board of Health*, 1907, 3, p. 38; by R. W. Doane *loc. cit.*, *infra*, and by myself in Oakland.

The following will illustrate very well how a flea population may once again come into its own in a locality where active sanitary measures are frowned upon. Recently a rat with acute septicemic plague was caught in the basement of a vacant dwelling-house right in the center of Oakland. The house faced the street; it had a vacant building on one side, a Japanese market on the other, and these were surrounded

below we have absolute evidence that squirrel fleas occur on rats and that squirrel fleas occur on and are known to bite humans. A case which is quite apropos, even though the evidence that the man acquired his infection from squirrels is not complete, might be cited here.

Mr. S., a resident of Oakland, who worked in the sewers, died July 21, 1908. He was attended by two prominent physicians who pronounced his disease "typhoid pneumonia." As his death occurred in four days after the onset, Passed Assistant Surgeon J. D. Long, then in charge of the work in Oakland, demanded an autopsy, which he performed himself at night in spite of the threats of a mob of the man's relatives and friends. The case proved to be one of pneumonic and septicemic plague and was proven such by bacteriologic examination and guinea-pig inoculation by myself and independently by Passed Assistant Surgeon McCoy of San Francisco and Drs. P. S. Newsbaumer and R. A. Archibald of Oakland, Cal. No plague rats had been found in Oakland since the middle of April. Still, as only 9,361 rats had been examined since the middle of April, it seemed possible that the man might have acquired the infection in the sewers. However, the Oakland council refused to appropriate money for further plague work on the basis of this case, though they had said, "Wait until we have human cases!" Some of the members of the council said that this was not a fair case, probably manufactured to influence them, and then, anyway, they had absolute evidence that the man had been hunting squirrels in Contra Costa County shortly before his illness. Taking it for granted that the man acquired his infection in Contra Costa, it was no fault of the town council that subsequent infection of rats did not occur. Owing to the great sanitary clean-up, human fleas were scarcer in the bay region than they had ever been as far back as native sons could recollect.

by perfectly filthy shacks occupied by Chinese. The basement was riddled by rat runways and rat droppings could be detected on the first and second floors. It was so heavily infested with fleas that the dust upon the floors could be seen to pulsate with their movements. Four sheets of fly paper were placed on the floor of the basement for one minute and then removed. One of these sheets was speckled with 190 fleas. Another sheet caught 115 fleas; a third about 95 fleas, and the fourth about 75 fleas. The legs of the man entering the house were covered with fleas and he was able to bottle 67 in a short time. The majority of these fleas were *P. irritans* but in addition there were five which, according to Passed Assistant Surgeon Carrol Fox, were probably introduced from neighboring chicken yards. This species has been taken from fowls, sparrow nests, and occasionally from rats and men.

As the house had a rat population only, these fleas must have derived their nourishment from rats, and at least one of these was plague infected. It would be simply marvelous if something did not happen with the elements in such favorable conjunction.

In August, 1908, R. W. Doane<sup>1</sup> of Stanford University, published a note on certain collections of fleas sent to him. Out of 174 fleas, collected by Dr. Snow from rats caught on the campus of the university there were eight ground-squirrel fleas. "The eight specimens, which seem to be identical with Baker's *Hoplopsyllus anomalus*, which was originally described from a spermophile in southern Colorado, are interesting in that they seem to show a possible connection between rats and squirrels. Dr. Blue has often stated that should the plague ever become endemic here, it would probably spread from the rats to the ground squirrels, thus making it much more difficult to stamp out."

That there is more than a possible connection between rats and ground squirrels is well known. Ranchers have often observed that, especially in harvest time, Norway rats emigrate into the fields and then may be seen running in and out of ground squirrel holes. During the past few weeks of September and October the men trapping ground squirrels for me in the Berkeley Hills succeeded in trapping more Norway rats (*Mus norvegicus*) than any other species of rodents. The game traps were set well within the burrows of *Otospermophilus beecheyi*. The occurrence of *Hoplopsyllus anomalus* on *Otospermophilus beecheyi* has already been pointed out by Passed Assistant Surgeon Carroll Fox.<sup>2</sup> And in September, 1908, Passed Assistant Surgeon Geo. W. McCoy proved experimentally that the *H. anomalus* and *Ceratophyllus acutus* removed directly from a squirrel would bite a human being and suck his blood, thus confirming the reports of ground-squirrel hunters that they are often severely bitten by squirrel fleas.

This report should impress others with the fact that while the meager corps of sanitary officers with which this government is supplied have been and are still wide awake to the gravity and necessities of the plague situation, much remains to be done. There is no question in my mind nor in that of many others that, in spite of the apparent disappearance of plague, it still lurks hidden in some lair, perhaps and probably in the subterranean burrows of the ground squirrel and only the right combination of circumstances is required for its reap-

<sup>1</sup> *The Canadian Entomologist*, August, 1908, p. 303.

<sup>2</sup> *Public Health Reports*, September 25, 1908, p. 1371.

pearance in epidemic form. Why not kill off the ground squirrels? It would require a well-trained army to do that. The only really efficient method of attacking them at present is with the so-called "gopher-smokers," by means of which they may be driven from their holes with carbon bisulphide or sulphur dioxide. This method is almost worthless in the dry season when the surface crust is rent by deep cracks; and in the wet season one is soon anchored to the earth by adobe mud. Dr. Currie found them immune to Danysz virus, Rattin, and Squirrelin. I have experimented with a bacillus which produces a pseudotuberculous disease in guinea-pigs.<sup>1</sup> Dr. Theobald Smith<sup>2</sup> once suggested the possible usefulness of this germ in the extermination of spermophiles. However, the results of my experiments up to date are not at all encouraging. Judging from observations made at autopsies on ground squirrels and from data collected from ranchers few squirrels will take a lethal dose of arsenic or phosphorus. However, experimental work along this line may bring forth fruit.

Dr. Blue has recommended to the State Board of Health that "one of the best methods of ridding an area infested with ground squirrels is to encourage their natural enemies, such as the weasel, skunk, fox, and hawk. It has been observed by the Biological Survey that districts infested by these predatory animals have only a few ground squirrels. That in places where they have been killed off the squirrels abound in great numbers."

Of course, if there were no rats, plague among squirrels would be of comparative insignificance owing to their less intimate association with humans. The formation of societies for the extermination of the rat in various parts of the world shows that the race is cognizant of the dangers from that source. It is universally conceded that all the methods of poisoning and trapping yield only a temporary reduction in the number of rats in any community; and the best known bacterial viruses fail to kill more than half the rats feeding upon them, and leave behind a large number of naturally or artificially immunized rats. Officers of the Indian Medical Service have made use of the

<sup>1</sup> *Infra*, p. 519.

<sup>2</sup> *Jour. Med. Research*, 1903, 9, p. 286.

cat, the natural enemy of the rat, in certain villages in the Punjab, India, with some measure of success. And more recently Dr. Robert Koch has expressed his opinion to the effect that the only solution lies in the breeding and maintenance of a race of cats which are born ratters.

It seems to me, as it does to many others, that the only way in which humans can hope to bring about a permanent reduction in the rat population of the world must be brought about by entirely different means. In this country at least state and local governments must pass and enforce alterations in our building laws so that structures already standing may be altered, and future buildings so constructed that rats may not gain access to foodstuffs or breeding-places within them, and so also not come into intimate association with humans inhabiting them. Work of this character has already been begun in several parts of the world, but heretofore has been purely spasmodic and of a local nature.

#### SUMMARY AND CONCLUSION.

The *Arctomyiinae* and *Sciurinae* are numerous, diversified, and widely distributed in North America. Plague among these animals is known elsewhere in the world. Following the epidemiological and experimental observations of officers of the United States Public Health and Marine Hospital Service the actual demonstration of plague among the California ground squirrels has been made. The relation between squirrel plague and the origin of certain human cases has been demonstrated. The pathologic and bacteriologic features of squirrel plague are discussed. The probable origin in squirrel plague of the successive outbreaks on the Pacific Coast is indicated. The intercommunication between ground squirrels, rats, humans, and their fleas is referred to, along with the difficulties and gravity of the problems which now face the Public Health Service of the United States.

In conclusion I wish to express my indebtedness to Passed Assistant Surgeon Rupert Blue, commanding, to Passed Assistant Surgeon J. D. Long, formerly in charge of Alameda and Contra Costa counties, and to Passed Assistant Surgeons Geo. W. McCoy and Carrol Fox

of the plague laboratory in San Francisco, for without their generous assistance and collaboration it would have been impossible to have presented much of the information embodied in this article.

DESCRIPTION OF PLATE 4.

PLATE.—Fig. 1, right lung and heart of squirrel No. 1; Fig. 2, three-fourths of spleen of squirrel No. 8. For descriptions see text.

PLATE 4.



FIG. 1.



FIG. 2.